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J. H. Longwell, *Director*

Reducing Spoilage In Shell Eggs By The Use Of Fungicides

MARTHA E. LORAH, E. M. FUNK, JAMES FORWARD

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Report on Department of Poultry Husbandry
Research Project 17, "Poultry Losses"

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MARTHA E. LORAH, E. M. FUNK AND JAMES FORWARD

INTRODUCTION

It is recognized that a large proportion of the spoilage in shell eggs is caused by the growth of microorganisms within the egg. Bacteria have been found to be the cause of black rots, green whites, soured eggs, and other types of spoilage. Molds have been observed and isolated from the outer shells, the air cells, and shell membranes of eggs; but little has been reported concerning their effect on the albumen and yolks.

The purpose of this investigation was three fold: First, to isolate and identify as many fungi as possible from eggs, making special note of the general appearance of the eggs from which they were isolated.

Second, to infect fresh laid eggs with a representative group of the fungi isolated (holding them in moist chambers at temperatures most suitable for mold growth) and to observe the gradual changes as the infection progressed.

Third, to study suitable fungicides that could be added to the egg shells or the egg processing oil to inhibit the growth of fungi on the shells, thus preventing internal quality deterioration.

REVIEW OF LITERATURE

Fungi have been observed in eggs for many years. Moran and Pique (1926) made a study of the effects of molds in eggs. Weston and Halnan (1927) reported that the genus Cladosporium caused black discoloration on shell membranes of eggs. Csontas (1928) found that 50 percent of the stale eggs examined in the Hungarian markets contained species of Sporotrichium which caused a gelatinous transformation of the albumen, and the colonies grew as patches on the shell membranes. He found that proper sanitation and lowered humidity decreased the incidence of Sporotrichium infection. James and Swenson (1929) found that Penicillium and Cladosporium caused black pin-point discolorations in eggs. In the Paris markets, Baeza (1934) found eggs infected with Fusarium, Penicillium, Hormodendrium, Cladosporium, Alternarium, Stempyhlium, Chaemtomium, and Cephalosporium. Sharp and Stewart (1936) and Tomkins (1937) discussed the prevalence of molds in storage eggs. Gisske (1937) stated that molds played an important part in spoilage of eggs and that the atmospheric mold population increased the incidence of mold infection in eggs.

Knowles and Clerkin (1938) investigated the extent of mold in sealed eggs in Ireland. They stated that outwardly normal eggs developed an average spoilage of about 12.5 percent as a result of molds in the air space and less on the shell membrane. The air space molds were all species of Penicillium while the others were enclosed in gelatinous knobs from which Sporotrichium was isolated. All possessed a musty odor. Fumigation decreased the amount of mold infection, particularly the Penicillia.

Simmonds (1938) reported that Shirlan AG proved effective for eggs under both cold storage and room temperature conditions. Ewell (1938) suggested the use of ozone in cold storage rooms for the control of mold growth. Haines (1939) reported to the Third International Congress for Microbiology that rotting in eggs was caused chiefly by mold and bacteria of the genera Pseudomonas and Proteus. Mallman and Michael (1940) made a thorough investigation of the types of fungi most frequently encountered in their area. They reported the finding of species in ten different genera. The Penicillia were the genus occurring most commonly on the eggs and the filler flats. They studied various fungicides and found sodium pentachlorophenate the most effective. They reported that the various phenol derivatives they used did not change the taste or odor of the eggs. Mallman and Michael stated that all molds were isolated from the inner surface of the shell, in the air sac, and from the chorio-allantoid membrane adjacent to the inner surface of the shell. They reported that at no time did the mold penetrate into the egg contents.

Miller (1940 and 1942) discussed the importance of propionic acid and its calcium and sodium salts as inhibitors of mold and bacteria in bakery and other food products.

Rosser and Associates (1942) studied the effectiveness of various sealing agents and fungicides on eggs to maintain shell quality at ordinary temperatures. They found that dimethyl urea was the most effective growth inhibitor for microorganisms and that vaseline was the best sealing agent. Satisfactory results were obtained by dipping the eggs in polyvinyl alcohol treated with dimethyl urea.

Tisdale and Flenner (1942) evaluated the efficiency of the thiuram sulfides and metal dithio-carbamates as fungicides. Palmiter and Hamilton (1942) demonstrated the effectiveness of ferric dimethyl dithio-carbamate as a spray to prevent scab on apples. Hearst and Hearst (1948) recommended the use of low molecular esters of p-hydroxy benzoic acid as fungicides to preserve eggs. Young (1948) successfully controlled mold rots (but not bacterial rots) by dipping eggs in a 0.5 percent solution of salicylanilide. Yushok and Romanoff (1949) found that eggs could be sealed by adding stearic, lactic, or acetic acid to the processing oil and by using certain plastics; but the incidence of mold growth increased. In 1950 they demonstrated the effectiveness of adding cetylpyridinium chloride to the lactic acid-oil

emulsion to prevent growth of fungi. Brown and Gibbons (1954) found Penicillium expansum most frequently in the air cells of eggs. They also isolated species of Sporotrichium, Cladosporium, Scopularopsis, and Wardomyces. They stated that mold growth was found occasionally in the air cells of oiled eggs, but rarely in the unoiled eggs.

IDENTIFICATION OF FUNGI

Procedure

Many cases of storage eggs have been broken out in the egg laboratory at the University of Missouri Poultry Farm during the last several years. General appearance, albumen score, and numerous other observations were made. At the same time, eggs that appeared abnormal were cultured on the standard media for growth of bacteria and fungi. Observations were made as to color of the mold growth, color adhering to the shell membrane, condition of the albumen, whether liquefied or gelled, presence of pin-point colonies in the albumen and, finally, the condition of the yolk.

The following were necessary to identify the cultures:

1. Isolation of pure culture.
2. Numerous transfers of the pure culture to study the various stages of growth.
3. Observations of the cultures as to growth habits, color of mycelium, sporing bodies and the reverse sides of the culture on a special mold medium.
4. Mounted slides for identification of the genus and further examination of the spores at higher magnification.

Pure cultures were obtained by the agar dilution method: Six tubes of potato dextrose agar were melted and then held in water bath at 42-43°C. A loop-full of mold culture was added to tube number one. This was thoroughly shaken and a loop-full of number one was added to number two. This was repeated until the six tubes were inoculated with decreasing amounts of the mold culture. These tubes were poured into sterile plates with labels and incubated at room temperature. After four or five days, single colonies of the various molds present, if mixed, were transferred to petri plates containing Czapek's medium, which was suggested by Thom[1] and others as being the best differentiating medium.

Mounted slides were prepared by tearing off a portion of the mold growth with a small amount of agar adhering to it and mounting it in a lactophenol solution to which methylene blue had been added.

Simple diagrams were made of the most commonly occurring genera of the fungi. They have been included in the appendix.

[1]Thom, Charles "The Penicillia," Williams and Wilkins Company; Baltimore, Maryland.

Data and Results

Some eggs were found which had spotty red discoloration on the shell membranes, and a pink albumen having the consistency of a stiff jelly. The yolks showed white crusting at the point of contact with the red spots on the shell membranes. Mold was isolated from both the crusted yolk and the shell membrane. Pure cultures from the mold were identified as *Penicillium roseo-purpureum,* using the system of Thom.

Numerous eggs were found that had blue-green liquefied albumen resembling that found in green whites caused by Pseudomonas fluorescens, but there was no fluorescence. The portion of the colonies that adhered to the shell membranes was rose colored. The eggs smelled very musty. Numerous yolks showed crusting. Pure cultures of this mold were found to be *Penicillium cyaneum.*

A few eggs had spots of orange on the shell membranes. In advance stages the albumen was completely liquefied and was colored a bright orange. Heavy crusts on the yolks were observed on most of the eggs. Pure cultures of this mold were identified as *Penicillium flavum.*

Eggs were observed that had olive colored growths in the air cells. Numerous pin-point colonies were dispersed throughout the gelled albumen. The eggs smelled very musty. Pure cultures of *Penicillium digitatum* were obtained from both the air cells and the white colonies in the albumen.

Pink to flesh colored colonies were observed on the shell membranes of numerous eggs. White pin-point colonies were floating throughout the yellow, liquefied albumen. Cultural findings placed this mold in the *Penicillium chrysogenum* group.

The portion of one mold colony found adhering to the shell membrane was yellow and that next to the albumen was red. The albumen was pink and gelled and contained white pin-point colonies dispersed throughout. Spot crusting was observed on the yolks. On Czapek's medium, the reverse side of the colonies was yellow while the surface growth was red with respiration droplets. Pure cultures of this mold, *Penicillium citreoroseum,* were obtained from the shell membranes, white pin-point colonies, and the crusted yolks.

During this investigation, numerous eggs were examined that had a distinct lemon-like odor when broken out. The liquefied albumen was a bright yellow. Green colonies were observed in the air cells and on the shell membranes. Yolks were crusted in the more advanced stages. The differentiating medium on which the cultures were grown was colored an intense yellow. The agent causing the color was found to be soluble in 70 percent ethyl alcohol. This mold was identified as *Penicillium citrinun.*

Bright green colonies in air cells and on the shell membranes were prevalent in storage eggs. In the three eggs, the albumen was intense green and

liquefied. The yolks were badly crusted. Repeated cultures and close microscopic examination placed these in a still different group. The penicilli were borne on short verticils or stems and showed much branching. This mold was identified as *Penicillium brevi-compactum.*

Brown spots were observed on the shell membranes of many eggs. Heavier brown growths were also in the air cells. In some instances the internal quality did not seem affected but if the colonies in the air cells were large or there were numerous spots on the shell membranes, then the yolks were crusted at the areas touching the brown discolorations. The albumen did not seem changed by this fungus. This was identified as *Penicillium commune,* a very common soil and air borne mold.

Penicillium expansum was isolated from numerous eggs having blue-green growths in the air cells or on the shell membranes. The albumen was gelled and a green pigmentation spread from the mold growth throughout the albumen. The tendency to show zone formation was evident in the eggs. The colonies on the shell membranes showed concentric rings. The areas between the rings were free of spores. This growth is typical for this mold.

Many eggs were observed where the molds showed the tendency, as previously described, of growing in concentric zones. However, the color of the colonies was a dark, leafy green in contrast with the blue-green. The albumen showed some gelling, and the yolks were crusted in many of the eggs. Pure cultures of *Penicillium glaucum* were isolated from these eggs.

Orange to sulfur yellow spots were observed on the shell membranes of a few eggs. The albumen was completely liquefied and colored a bright yellow. The yolks were crusted at the point of contact with the shell membranes. This mold was identified as *Penicillium sulphureum.*

One of the molds occurring most commonly in egg spoilage was *Aspergillus niger.* The air cells were filled with purple black growths. Large purple colonies adhered to the shell membranes. The spore heads visible with the hand lens were large and globose. The albumen was nearly solid, and the yolks showed crusting in nearly all the eggs in which *Aspergillus niger* was found.

Numerous eggs were examined that had a dark green to nearly black growth in the air cells. In two of the eggs with the heaviest growth, the albumen was thickly gelled; and the yolks were crusted. Pure cultures of this mold were found to be *Aspergillus fumigatus.*

Several eggs that were opened had large green growths in the air cells. The stalks on which the spore heads were borne were much longer than those described previously and were visible with the hand lens. These were identified as *Aspergillus giganteus.*

A few of the eggs examined had orange growths in the air cells and on the shell membranes. The albumen was liquefied and colored orange.

The yolks showed much crusting. *Aspergillus ochraceus* was found in this type of spoilage.

Many eggs were observed that had grey-green growth in the air cells. The albumen was orange and had the consistency of a thick jelly. In some cases the yolks appeared cooked and resembled ping pong balls. The *Aspergillus glaucus* group was present in these eggs.

Aspergillus nidulans was isolated from three eggs in which a blue-green growth was found in the air cells. The colonies adhering to the shell membranes were red. The albumen was congealed, and crusting was present on each yolk. Huelle cells were observed on the slide preparation of both of the cultures taken from these eggs.

Conspicuous ivy green growth was observed in the air cells of a few eggs. The pale green albumen was thick and jelly-like. *Aspergillus oryzae* group was the mold obtained in pure culture from these eggs.

Aspergillus clavatus was isolated from several eggs having blue-green colonies in the air cells. The albumen was liquefied, and the yolks showed crusting. The clavate spore heads were borne on fairly long stalks.

Four eggs were opened that had a putrid odor, compared to the musty odor of many moldy eggs. The growth in the air cells was buff brown, the albumen was liquefied and colored orange-red, and the yolks were badly crusted. On Czapek's medium the colonies were nearly black. The conidiophores, or spore stalks, which were pale yellow, terminated in subglobose to elliptical vesicles. The spore heads were columnar. Orange-red respiration droplets were dotted over the mycelial growth. The odor of the growing cultures was quite putrid and was similar to the odor of the eggs. The cultures were identified as *Aspergillus flavipes* according to the classification of Thom and Church.[2]

Numerous eggs were badly infected in the air cell end with *Aspergillus terreus,* a common fungus of the soil. The colonies on the shell membranes were brown. The albumen was congealed, and the yolks were crusted.

Eggs were opened that had the entire air cells filled with grey-black growth. Upon culturing, this growth was quickly classed as a Rhizopus because of the presence of hair-like projections that fell over on the culture medium and reproduced. The stalks were erect, bearing globose black spore heads which were quite large. *Rhizopus nigricans,* as this was designated, was often found in cracked eggs or in eggs with poor shells.

Mucor spinosus and *Mucor pyriformis* were found in several eggs. Grey to dirty brown growths nearly filled the air cells. The gelled albumen was colored a light brown. Yolks were badly crusted in all the eggs.

[2]Thom, Charles and Margaret Church—The Aspergilli, Williams and Wilkins, Baltimore, Maryland.

Cladosporium herbarum was found in many eggs that had black pinpoint colonies on the shell membranes. In the later stages of infection, the albumen was congealed; and the yolks were crusted.

Numerous eggs that were cultured had brown cotton-like growth in the air cells. The albumen was liquefied, the yolks were crusted, and a peculiar cabbage-like odor was detected in each egg. This group was identified as *Scopulariopis brevicaulis.*

Gelatinous knobs were noticed on many eggs, particularly dirty eggs that had been held in storage. These knobs were plated on potato dextrose agar and found to have numerous species of the*Sporotrichium* group in them.

Two eggs were broken out that had heavy grey growth in their air cells. The albumen was congealed, and the yolks were crusted. Upon culturing, the colonies grew with a heavy grey felt. The two-celled spore heads were borne on short stalks. This fungus was identified as *Trichothecium roseum.*

Several eggs were opened that had white powdery growth in the air cells and brown discolorations on the shell membranes. Cultures made from these were identified as *Oospora lactis.*

Alternarium tenuis was found in many eggs that were inedible. The air cells were filled with a heavy grey growth. The albumen was completely gelled, and the yolks were badly crusted.

At various times eggs were opened that had pink liquefied albumen, and the yolks appeared much larger than usual and were dark and watery. The mold grew readily on potato dextrose agar. In about a week, the upper surface of the growth of mycelium was white and cottony while the reverse was a bright red. This was identified as the *Actinomycetes ruber* group.

Another species of the *Actinomycetes* group caused marked orange liquefaction of the albumen and enlargement of the yolk. The reverse of the mycelial growth on the culture plates was orange.

INFECTION OF FRESH EGGS

Procedure

Many different fungi were isolated from spoiled eggs. To prove that these fungi were responsible for the spoilage, it was necessary, first, to try to reproduce the spoilage by inoculation of fresh, presumably sterile, eggs with a representative group of the fungi from spoiled eggs. Second, if spoilage did occur it was necessary to recover the fungi in pure culture from the inoculated eggs.

To do this, moist chambers were set up, consisting of wide-mouth gallon jars in which the eggs could be held in an extremely humid atmosphere at 70-75° F. Stones were put in the bottom of the jars and nearly covered with water. The jars were sterilized in the autoclave for 20 minutes at 15 pounds pressure.

The test fungi were grown on potato-dextrose agar. After five days the cultures were washed with sterile nutrient broth, and the washings were swabbed on the surfaces of 12 fresh eggs, the number of eggs used for each test fungus. The eggs were immediately placed in the sterile jars. Twelve control eggs were held in sterile jars without inoculation. The humidity was not measured, but it was very high as droplets of water formed on the sides of the jars and on the eggs. During the first and second weeks, the eggs were candled; and visible changes were noted. Four eggs were broken out on the third week and cultured on potato-dextrose agar. The remaining eight eggs were cultured at the end of the experiment.

Results

Penicillium cyaneum:

First Week: Shell—Moderate white to green growth.
Second Week: Shell—Heavy blue-green growth.
Third Week; Four Eggs Opened: Shell Membranes—Rose spots.
Air Cell—Blue green growth.
Culture—Penicillium cyaneum found in each egg.
Fourth Week—Penicillium cyaneum was found in all eight eggs.

Penicillium flavum:

First Week: Shell—White to green mycelia growth.
Second Week: Shell—Pale green growth.
Candling—dark spots on shell membrane.
Third Week—Green growth in air cells.
Spots on membranes.
Penicillium flavum was present.
Fourth Week—Heavy green growth in air cells.
Orange spots on shell membranes.
Albumen was liquefied and orange in color.
Twelve eggs were inedible.
Penicillium flavum was isolated from each.

Penicillium citrinun:

First Week: Shell—Pale blue-green mycelial growth.
Second Week—Pin-point colonies visible on shell membranes with the aid of the candling light.
Third Week: Four eggs were opened.
Blue-green growth in air cells.
Lemon-colored albumen. Crusted yolks.
Penicillium citrinun was present.
Fourth Week—Eight eggs were inedible.
Heavy blue-green colonies in air cells.
Albumen was liquefied and lemon colored.

Yolks were crusted.
Penicillium citrinun was isolated.

Aspergillus glaucus:
First Week: Shell—Pale green growth.
Second Week: Shell—Heavy green growth.
Candling—Pin-point colonies in air cells and on membranes.
Third Week—Green colonies were on membranes and air cells.
White pin-point colonies dispersed throughout the thick albumen.
Aspergillus glaucus was isolated.
Fourth Week—Eight eggs were inedible.
Heavy green growth in air cells.
Albumen was congealed and yolks were crusted.
Aspergillus glaucus was found in each egg.

Aspergillus niger:
First Week: Shell—Grey growth
Second Week—Dark colonies on membranes and air cells.
Third Week —Twelve eggs were inedible.
Purple-black colonies were in the air cells and on the shell membranes.
Albumen was completely congealed.
The yolks were crusted.
Aspergilus niger was found in each egg.

Rhizopus nigricans:
First Week: Shell—Grey growth.
Second Week: Shell—Heavy grey growth.
Candling—Penetration of hyphae through large pores.
Black spots on membranes.
Third Week; Four eggs opened —
Black pin-point colonies on shell membranes.
Albumen was congealed; yolks were crusted.
Rhizopus nigricans was isolated.
Fourth Week—Eight eggs were inedible.
Shell membranes were dotted with black colonies.
Spore heads were visible without magnification.
Albumen was gelled and all yolks were crusted.
Rhizopus nigricans was isolated from each.

Actinomycetes ruber:
First Week: Shell—White mycelial growth.
Second Week: Shell—Pale pink mycelial growth.

Third Week; Four eggs opened —
White colonies in the air cells; pin-point colonies in the albumen.
Actinomycetes ruber was isolated.
Fourth Week; Eight eggs were opened—
White mycelial growth on the shell membranes with the portion touching the membrane being red.
The yolks were enlarged and watery, sometimes accompanied by crusting.
Actinomycetes ruber was isolated from the inedible eggs.

Alternarium tenuis:
First Week: Shell—Grey cottony growth.
Second Week: Shell—Heavy grey growth.
Candling—Dark pin-point colonies on membranes.
Third Week—Twelve eggs were opened and found inedible.
Heavy grey-black growth in air cells. Membranes were dotted with dark spots. Albumen was congealed.
Yolks were crusted.
Alternarium tenuis was found in each.

Controls; no inoculation; held in sterile container:
First Week—No growth.
Second Week—No growth.
Third Week—Faint white mold growth on shell surface.
Fourth Week—One of the 24 eggs had a small green colony in the air cell when the eggs were broken. It was identified as *Penicillium expansum,* which is one of the common soil and air fungi, which perhaps came from handling the eggs. No fungi were found within the other eggs. All were edible.

By watching this gradual infection of eggs with a representative group of fungi, conclusive proof was obtained that molds caused spoilage in eggs not only affecting the air cells and shell membranes; but if eggs were held long enough, they caused breakdown of the albumen and abnormal changes in the yolks. Eggs that showed colonies in the air cells and on the shell membranes were considered inedible as the albumen and yolks harbored spores which developed, causing deterioration of the eggs. Mustiness usually accompanied the mold growth in the eggs even though marked changes had not occurred in the eggs.

Upon examining the control eggs, it was evident that clean appearing fresh eggs harbored some mold spores since after three weeks in an unopened moist chamber a faint white growth became visible on the shells. During the limits of these experiments, only one of the 24 eggs showed mold within the egg.

In conclusion, fungi will cause spoilage in eggs if they are held long enough for the fungi to penetrate into the interior of the egg. Spoilage appears as air cell colonies, discoloration of shell membranes where colonies are present, crusting or enlargement of the yolks, liquefaction or gelation of the albumen and, usually, mustiness of the entire egg, causing bad flavor which is easily imparted to eggs nearby.

TESTING OF FUNGICIDES

Mold spores are ever present in the soil and air. Eggs, even though they appear clean, harbor spores on the shells. Eggs, when held in storage, are sometimes in an atmosphere which is conducive to mold growth. The preceding work shows that there are many types of molds that cause loss in eggs, and something should be done to reduce them to a minimum. Most storage eggs are oiled before they are placed in storage. The slightly oily shells make an ideal surface for molds to collect and grow. If conditions are suitable, the spores first produce a minute growth; and, if sufficient time elapses, the mold penetrates the shell, the membrane, and finally the egg meat, thus resulting in loss of the egg.

The question arose whether suitable fungicides could be found to add to the egg shell or to the processing oil to inhibit mold growth on the shells.

Procedure

The general procedure for testing various fungicides was as follows:

Suitable mold cultures were supplied that had been isolated from eggs and could be washed off to get a suspension of mold spores to use as culture or inoculum for the eggs.

Moist chambers were prepared. These may consist of a desiccator with water in the bottom, a large jar with stones in the bottom nearly covered with water, or for a larger container, an Arnold sterilizer may be used. The Arnold sterilizer is a double jacketed container with water in the lower part to maintain a constant humidity. The moist chambers were set up 4 to 5 days before experiments were to be started and sprayed with a suitable mold culture, sometimes with mixed cultures.

Eggs were usually held at room temperature and weekly observations, consisting of the following, were made:

First week-Shell condition was noted. Eggs were candled.

Second week-Appearance of shell surface and the candling data were recorded. Part of the eggs in each lot were opened and cultured on potato-dextrose agar.

Third and fourth week—The procedure of the second week was repeated until all of the eggs were opened and cultured.

Results

Zinc dimethyl dithio-carbamate: The carbamates have been shown to be effective in spraying fruit trees and certain vegetables to control mold

growth, and it seemed possible to use them on storage eggs. The iron salt could not be used as it was dark brown and even in the minute concentrations that were necessary, discolored the eggs shells when it was applied to them. The zinc salt was chosen, and E. I. duPont de Nemours and Company[3] supplied a sample, In3665A25, under the trade name "Milban" (zinc dimethyl dithio-carbamate). It is a white powder which forms a workable emulsion in mineral oil, the type used for egg processing. Ten grams of zinc dimethyl dithio-carbamate were ground in a mortar, and small amounts of egg processing oil were added to make a thick paste. Oil was added until 100 milliters of emulsion were prepared. This made a 10 percent oil emulsion which was used as the stock. Thorough mixing was necessary before it was further diluted for use as a dip for the eggs. A 10 percent aqueous solution was made by grinding 10 grams of zinc dimethyl dithio-carbamate in a mortar and adding enough water to make 100 milliters of solution.

A preliminary run was made as follows: A desiccator with water in the bottom was used as a moist chamber. It was sprayed with a mixture of mold spores and allowed to stand, closed, at room temperature for several days. Droplets of water formed inside of the cover and on the sides, suggesting nearly 100 percent humidity. Mold growth was visible on the walls of the desiccator. Four eggs were dipped in a 1 percent oil emulsion of zinc dimethyl dithio-carbamate made by diluting the 10 percent stock emulsion. Four eggs were dipped in clear oil which did not contain fungicide. These eggs were held in the moist chamber at room temperature, which varied from 70 to 80°F. Observations and results of the test are given in Table 1.

TABLE 1--THE EFFECT OF 1% ZINC DIMETHYL DITHIO-CARBAMATE IN OIL ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94 TO 99% RELATIVE HUMIDITY

Time Held	Observations	
	Treated	Controls
1 Week	No visible growth	White mold on shells
2 Weeks	No Visible growth clear by candle-light	Blue green on shells Pin-point colonies in air cells
3 Weeks	4 negative	4 positive and inedible

In all of the tables, the positive or negative signs refer to growth or no growth, respectively, on the culture plates.

At the higher temperatures, embryonic development made it harder to get an accurate interpretation of the changes in the yolk; so throughout most of the following experiments part or all of the eggs were thermostabilized at 130° F. for 15 minutes. The experiment was repeated, thermo-

[3]E. I. duPont de Nemours and Company, Wilmington, Delaware.

stabilizing 24 eggs in water. Twelve were dipped in oil containing 1 percent zinc dimethyl dithio-carbamate and 12 were dipped in clear oil. The eggs were held for three weeks in a large moist chamber (Arnold sterilizer). Results are given in Table 2.

TABLE 2--THE EFFECT OF 1% ZINC DIMETHYL DITHIO-CARBAMATE IN OIL ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY

Time Held	Treated Eggs	Control Eggs
1 week	No visible growth	White mold on shells
2 weeks	No visible growth Normal with the candlelight	Pin-point colonies in air cells
3 weeks	12 negative cultures	12 Positive cultures
Condition of albumen at end of 3 weeks	Score 1.54 Height 6.63	12 inedible eggs

These experiments using the 1 percent zinc salt were repeated several times, and a summary of those results is given in Table 3.

TABLE 3--THE EFFECT OF 1% ZINC DIMETHYL DITHIO-CARBAMATE IN OIL ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY

Total Number of eggs	Fungicide Treated Eggs	Control Eggs
186	180 Negative 6 cracked and positive	174 Positive 6 negative 6 cracked

From time to time, photographs were taken of the eggs when they were taken out of the moist chambers. In Figure 1, the control eggs, labelled 4CO, were partially covered by a mold growth. The eggs (4 AO) dipped in the zinc salt were free of mold growth.

It was evident from the tabular results and the photographs that 1 percent zinc dimethyl dithio-carbamate was an effective fungicide to add to oil for the processing of eggs. The next question was whether lesser concentrations would be effective.

A preliminary test with lesser concentrations was made with four eggs in each lot receiving the following treatment:

A. Thermostabilized and dipped in 0.25 percent zinc salt in oil.

B. Thermostabilized and dipped in 0.5 percent zinc salt in oil.

C. Thermostabilized and dipped in clear oil.

These were held for two weeks at room temperature in a desiccator serving as a moist chamber containing mold spores. At the end of the two week period, the eggs were opened and cultured. The results are given in Table 4.

Fig. 1—Oil processed controls on the left are covered with mold. Eggs on the right were processed in oil containing 1 percent zinc dimethyl dithiocarbamate.

TABLE 4--THE EFFECT OF VARIOUS CONCENTRATIONS OF ZINC DIMETHYL DITHIO-CARBAMATE IN OIL ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY

Time Held	Fungicide Concentrations 0.25%	0.5%	Control Eggs
1 week	no growth on shells	no growth	mold on shells
2 weeks	no growth on shells	no growth	Heavy growth on shells
3 weeks culture	4 negative	4 negative	4 positive

With this information it was decided to prepare 30-egg lots, using 1.0 percent, 0.5 percent, 0.25 percent, and 0.1 percent concentrations of zinc dimethyl dithio-carbamate. The lots were arranged as follows:

30 eggs —No thermostabilization and no oiling.
30 eggs—Thermostabilized and dipped in clear oil.
30 eggs—Thermostabilized and dipped in oil-1 percent zinc salt.
30 eggs—Thermostabilized and dipped in oil-0.5 percent zinc salt.
30 eggs —Thermostabilized and dipped in oil-0.25 percent zinc salt.
30 eggs—Thermostabilized and dipped in oil-0.1 percent zinc salt.

Five eggs in each lot were placed in six layers (three on each side of the case). The case was held at 61° F. with a humidity of 95-96 percent. The results are given in Table 5.

Numerous experiments were completed repeating this work. The results are given in Table 6. Tables 5 and 6 demonstrated the effectiveness of the lower concentrations of zinc dimethyl dithio-carbamate.

Zinc dimethyl dithio-carbamate was used throughout these experiments in an oil emulsion. This seemed advisable as the oil for egg processing can be a source of infection. The next question was, could the fungicide be placed on the egg before it was dipped in oil; and protect it from mold infection. Experiments were carried out on three occasions using three concentrations (1, 0.5, and 0.25 percent) of aqueous zinc dimethyl dithio-carbamate, prepared from the 10 percent aqueous stock solution. Eggs were first thermostabilized for 15 minutes in 130° F. water, dipped in various concentrations, allowed to dry and then dipped in a clear oil. They were held at room temperature for one month in an Arnold sterilizer. Results from these three experiments are given in Table 7. Zinc dimethyl dithio-carbamate was an effective fungicide when used as an aqueous dip before oiling the eggs.

Tetra-ethylthiuram monosulfide: Since this material had been used successfully as a fruit spray, it was selected for another trial in egg processing.

TABLE 5--THE EFFECT OF VARIOUS CONCENTRATIONS OF ZINC DIMETHYL DITHIO-CARBAMATE IN OIL ON PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY

Time Held	Control Eggs	Oil Only	1%	0.5%	0.25%	0.10%
2 Weeks						
Shell	5+	5+	5-	5-	5-	5-
Shell membrane	1+	2+	5-	5-	5-	5-
Yolk	1 mottled	2 mottled				
Alb. score	3.43	6.65	6.58	7.31	5.61	7.28
Alb. height	3.3	2.0	1.9	1.4	2.3	1.7
3 Weeks (10 eggs)						
Shell	10+	10+	10-	10-	10-	10-
Shell Membrane	4+	5+	10-	10-	10-	10-
Yolk	1 ined.	4 ined.				
Alb. Score	3.58	5.98	6.14	6.78	6.15	5.49
Alb. Height	3.2	1.9	2.1	1.8	1.9	2.0
4 Weeks (15 eggs)						
Cultures	15+ 13 ined.	15+ 13 ined.	13- 2 cracked	13- 2 cracked	12- 2 cracked	15-
Alb. Score	2.5	3.48	7.06	7.67	7.18	6.97
Alb. Height	4.0 (2 egg samp.)	4.0 (2 egg samp.)	2.1	2.0	2.0	2.0

TABLE 6--THE EFFECT OF ZINC DIMETHYL DITHIO-CARBAMATE ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70° F. - 80° F. AND 94-99% RELATIVE HUMIDITY FOR APPROXIMATELY ONE MONTH

No. of eggs per lot	0.5% in oil		Controls	
	15 min. at 130°F.	No stabilization	15 min. at 130°F.	No stabilization
12	1+ 11 neg.	12 neg	6+ 6 neg.	11+ 1 neg.
12	12 neg.	12 neg.	11+	12+
20	20 neg.	20 neg.	16+	20+
9	9 neg.	9 neg.	9+	9+
30	30 neg.	28 neg. 2+ shell memb.	30+	30+
		0.25% in oil		
30		1 cracked ined. 29 neg.	30+ all ined.	
		0.10% in oil		
30		30 neg.	30+ all ined.	

TABLE 7--THE EFFECT OF AQUEOUS SOLUTIONS OF ZINC DIMETHYL DITHIO-CARBAMATE ON PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY

Number of eggs in lots	1%	0.5%	0.25%	Control
15	15-	15-	15-	15+ and inedible
30	30-	30-	30-	30+ and inedible
30	29- 1 cracked +	30-	28- 2+	30+ and inedible

A 1 percent oil emulsion was prepared by diluting 10 ml. of a 90 percent tetra-ethylthiuram monosulfide to 900 ml. (supplied by the E. I. du Pont de Nemours and Company) of oil suitable for egg processing. The 1 percent emulsion was further diluted to 0.5 percent with oil. Even though these emulsions were slightly colored they did not discolor eggs.

Moist chambers for holding the eggs during the experiments were similar to those described in the general procedure.

Results of the work which showed the effectiveness of tetra-ethylthiuram monosulfide as a fungicide are given in Table 8. Photographs were made of the eggs when they were taken out of the moist chambers, and Figure 2 was chosen as the one which most clearly showed the appearance of the eggs. The two eggs on the left of the picture did not receive tetra-ethylthiuram monosulfide, and they are heavily infected with mold. The two eggs on the right were dipped in the oil containing 1 percent tetra-ethylthiuram monosulfide. They were free of mold growth.

TABLE 8--THE EFFECT OF TETRA-ETHYL THIURAM MONOSULFIDE IN OIL ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY FOR APPROXIMATELY ONE MONTH

Number of eggs per lot	0.5% in oil		1% in oil		Controls	
	Stabilized 15 min. at 130°F.	Not Stab.	Stabilized 15 min. at 130°F.	Not Stab.	Stab. 15 min. at 130°F.	Not Stab.
12	2+	8+			11+	12+
	10-	4-			1-	
20			19-	19-	16+	20+
			1+	1+	4-	
9				9-		9+
15			12-	12-	15+	15+
			3+	3+		

From Table 8 and Figure 2, it can be concluded that tetra-ethylthiuram monosulfide is an effective fungicide to use in egg processing oil. Since a slight odor was detected in the freshly broken out eggs, this fungicide might seem unsuitable; but it must be remembered that these eggs were held in sealed containers. Those not so tightly sealed did not pick up the odor of the fungicide. The 0.5 percent emulsion was not quite as effective, but these eggs were all subjected to the most favorable conditions for fungal growth.

Thiourea (thio-carbamate): The work of Rosser and associates in Canada showed that dimethyl urea was effective as a fungicide for eggs, but they did not make use of thiourea. Since it is a good fungicide for other products, it was thought worthwhile to try its effects on storage eggs. Oil emulsions containing 1.0 percent and 0.5 percent of thiourea were prepared. Two experiments were carried out using the 1.0 percent and four using the 0.5 percent thiourea in oil. The general procedure for setting up the experiments and for holding the eggs in a moist chamber at room temperature was followed. Results of the various experiments are given in Table 9.

TABLE 9--THE EFFECT OF THIO-UREA IN OIL ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70° F.-80° F. AND 94-99% RELATIVE HUMIDITY

No. of eggs per lot	0.5% in oil		1.0% in oil	Controls	
	15 min. at 130°F.	No stabilization	No stabilization	15 min. at 130°F.	No stabilization
12		12 neg.	1 cracked + 11 neg.		12+
26			No ined. 6+ 20-		26 ined.
30	28 neg. 2+			26 ined. 4 neg.	30 ined.
30	3 shell memb. + 27 neg.			30 ined.	
30	29 neg. 1 ined. (E. coli)			27+ (13 ined.) 3 neg.	
50	45 neg. 4+ (crusted) 1 ined.			40 ined. 4+ shell memb. 4+ Air cell 2 neg.	

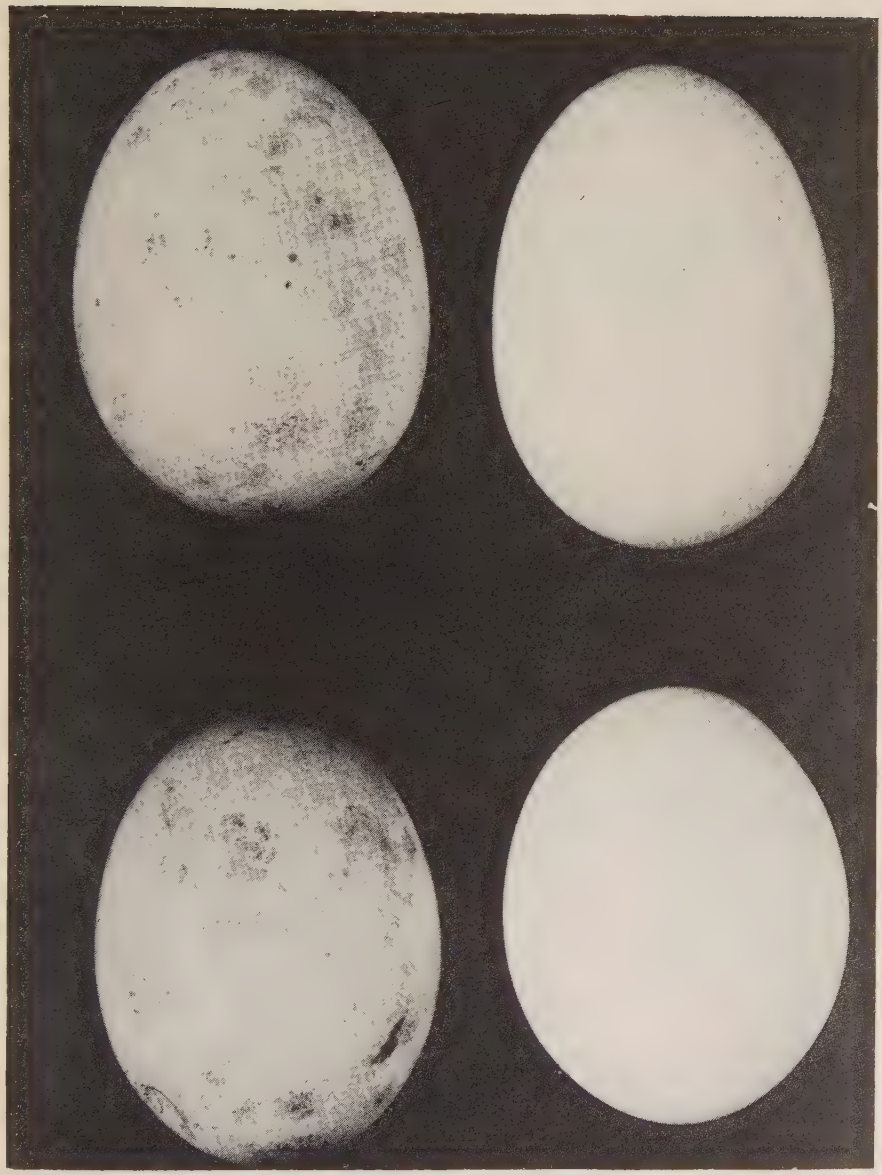


Fig. 2—Oiled controls on the left are covered with mold. Eggs on the right were processed in oil containing 1 percent tetra-ethylthiuram monosulfide.

Photographs were taken of the eggs as they were removed from the moist chambers. Figure 3 illustrates the appearance of the eggs. The "C" egg was the control receiving no fungicide, and the "B" egg had been dipped in 0.5 percent emulsion of thiourea in oil. Figure 4 shows the penetration of the mold to the membrane and on the yolk as crusting in the control egg.

One, 0.5, and 0.25 percent aqueous suspensions of thiourea were used as dips before oiling in three different experiments. The results of those experiments are given in Table 10.

TABLE 10--THE EFFECT OF THIOUREA IN AQUEOUS SOLUTION AS A DIP BEFORE OILING ON THE PREVENTION OF MOLD ON SHELL EGGS HELD APPROXIMATELY ONE MONTH AT 70-80° F. AND 94-99% RELATIVE HUMIDITY

No. of Eggs	1%	0.5%	0.25%	Controls
12	12-	12-	12-	12+(inedible)
30	30-	30-	27 neg. 3-on shells	30+(inedible)
30	30-	30-	29- 1+(shell memb.)	30+(inedible)

Thiourea proved to be an excellent fungicide to use on eggs. Both the oil emulsion and the aqueous suspensions were good, even in the lower concentrations. The eggs dipped in aqueous suspensions, followed by oil processing, appeared to have the best protection. The eggs were not discolored, and there was no odor when the eggs were broken out. Since it is described in Merck Index Number 5 as a non-toxic substance, thiourea should prove to be a suitable fungicide to use on eggs before placing them in storage.

Dowicide G: Since the work of Mallman and co-workers suggested that sodium pentachlorophenol[4] was an effective fungicide on egg cases and fillers, it was another substance chosen for trial on eggs.

One, 0.5, and 0.25 percent oil emulsions were prepared by diluting the sodium pentachlorophenol[4] crystals with eggs processing oil. The vapor from the salt proved to be an irritant to the respiratory tract of the person making the emulsions. Eggs were dipped in these concentrations and held in an atmosphere with high mold population and a relative humidity approaching 100 percent. Results are in Table 11.

TABLE 11--THE EFFECT OF DOWICIDE G* IN OIL ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY FOR ONE MONTH

Number of eggs per lot	0.25% in oil		0.5% in oil		Controls	
	15 min. at 130°F.	Not Stab.	15 min. at 130°F.	Not Stab.	15 min. at 130°F.	Not Stab.
12	2+	4+			6+	11+
	10-	8-			6-	1-
12	3+	6+			11+	12+
	9-	6-			1-	
20			2+	4+	16+	20+
			18-	16-	4-	

* Supplied by the Dow Chemical Company, Midland, Michigan

[4]Dow Chemical Company, Midland, Michigan.

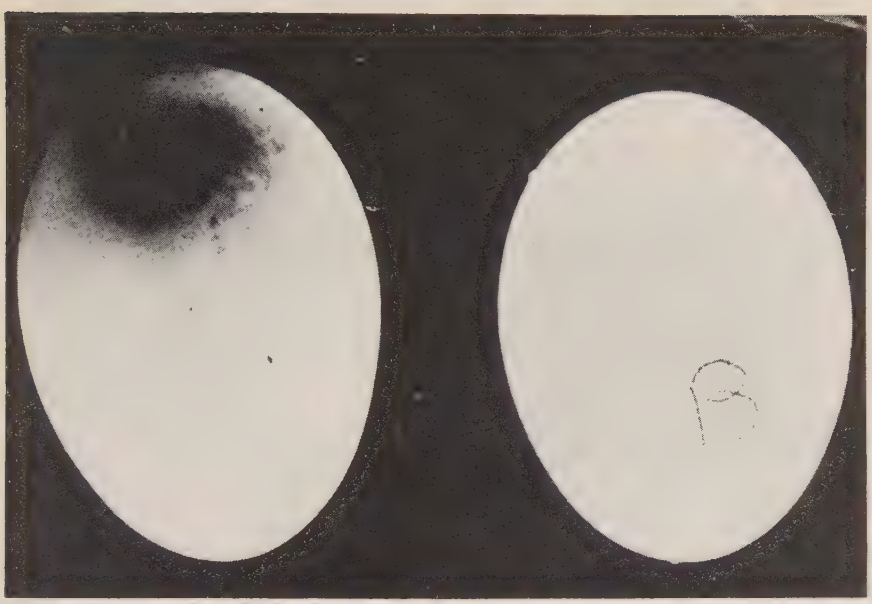


Fig. 3—The effect of Thiourea in preventing mold growth in shell eggs. (C) Control, (B) 0.5 percent thiourea in oil.

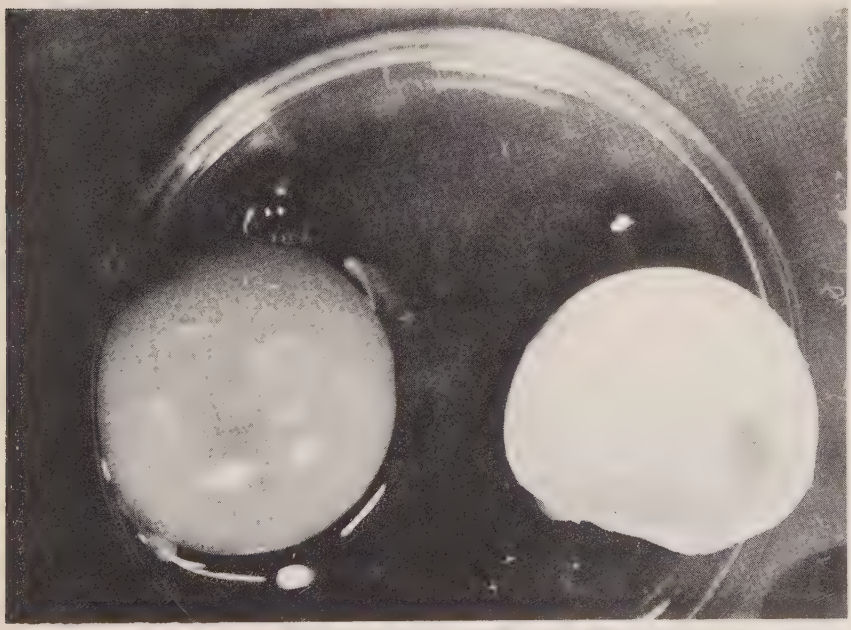


Fig. 4—The development of mold on the shell membrane and on the yolk of eggs. Left, crusted yolk. Right, mold on membrane.

At the end of the experiments, the broken out eggs retained a slight odor of the sodium pentachlorophenol. This substance had some fungicidal powers, but its use did not seem warranted because of its irritating effect on the respiratory tract and the slight odor in the eggs.

Alkyl Dimethyl Benzyl Ammonium Chloride: The use of quaternaries for bactericides and fungicides has expanded rapidly. Alkyl dimethyl benzyl ammonium chloride, under the trade name BTC[5] was chosen as one of the quaternaries most easily obtained. Various concentrations were prepared from the 50 percent BTC that was supplied. The oil emulsions were made more stable with the addition of a few drops of a wetting agent, "Neutronyx 600"[6]. Experiments were designed to use various concentrations. Results are given in Table 12. Figure 5 illustrates the appearance of the eggs at the

TABLE 12--THE EFFECT OF BTC* (ALKYL DIMETHYL BENZYL AMMONIUM CHLORIDE) IN OIL ON THE PREVENTION OF MOLD ON THERMO-STABILIZED SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY FOR ONE MONTH

Number of eggs per lot	% of BTC in oil			Controls
	0.1%	0.01%	0.001%	
10	10-			10+ and inedible
30	30-	25- 4+ mold 1 E. coli	19- 11+	27+ and inedible
20	20-	17- 3+	15- 5+	20+ and inedible

* Supplied by Onyx Chemical Company - Jersey City, New Jersey

end of the experiments. The two eggs labelled A were clean eggs dipped in oil only. They were heaving coated with mold growth. The two eggs labeled B were clean eggs washed in an alkaline detergent and dipped in oil only. They were also overgrown with mold. The two eggs labelled C were clean eggs dipped in oil containing 1 tablespoon of 50 percent BTC per 1 gallon of oil. "BTC", in the concentrations used proved to be a good fungicide. Eggs dipped in various oil emulsions of BTC held for a month under very adverse conditions were excellent as compared to the controls that were inedible. No odor was detected in the eggs.

Sodium propionate: Since sodium propionate has been used effectively in bread and cheese to inhibit mold growth during storage, it was included in the experiments on eggs.

A 1 percent sodium propionate-oil emulsion was prepared. The treated and control eggs were held in a moist chamber for a month at 70-75° F. Results are given in Table 13.

[5]Onyx Chemical Company, Jersey City, New Jersey.

[6]Onyx Chemical Company, Jersey City, New Jersey.

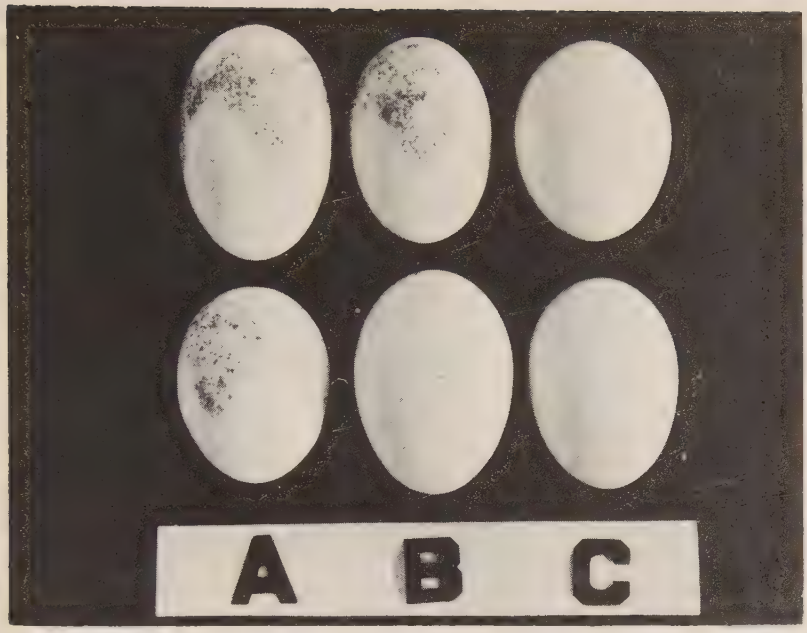


Fig. 5—Effect of a quaternary in preventing mold growth. (A) Clear oil; (B) Alkaline detergent and oil; (C) BTC in oil.

TABLE 13--THE EFFECT OF 1% SODIUM PROPIONATE IN OIL ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-99% RELATIVE HUMIDITY FOR ONE MONTH

Number of eggs	Sodium propionate in oil	Clear oil
24	17-	23+
	7+	1-

One percent sodium propionate in oil had only partial fungicidal value. Since 2 to 4 percent concentrations are used in food wrappers, a 4 percent aqueous solution was prepared for trial as a dip before oiling the eggs. Ninety-six eggs were treated in this manner and held along with 24 eggs dipped in oil only, using an Arnold sterilizer as a moist chamber for one month at 70-75° F. Results are given in Table 14.

TABLE 14--THE EFFECT OF 4% AQUEOUS SODIUM PROPIONATE AS A DIP BEFORE OILING ON THE PREVENTION OF MOLD ON SHELL EGGS HELD AT 70-80° F. AND 94-90% RELATIVE HUMIDITY FOR ONE MONTH

	Sodium propionate dip followed by oil	Oil Only
Number of eggs	96	24
Condition of eggs	All edible	All edible
Culture	94-	24+
	2+	

The results showed that a 4 percent aqueous sodium propionate solution had excellent fungicidal value if used on eggs before they were oiled.

SUMMARY

1. Eggs are subject to mold infection if atmospheric conditions are conducive to fungal growth. Numerous species of Penicillium, Aspergillus, Rhizopus, Mucor, Cladosporium, Trichothecium, Oidium, Alternarium, and Actinomycetes were isolated from storage eggs.

2. Eggs were reinfected with eight of the fungi-isolated from the storage eggs. Infections similar to those in the eggs from which they were isolated were produced in the inoculated eggs. Pure cultures of the inoculum were obtained from the eggs.

3. Zinc dimethyl dithiocarbamate was effective as a fungicide, in both aqueous solutions and oil emulsions in 1.0 to 0.1 percent concentrations.

4. Tetra-ethylthiuram monosulfide was effective in 1.0 percent concentration but less effective in 0.5 percent.

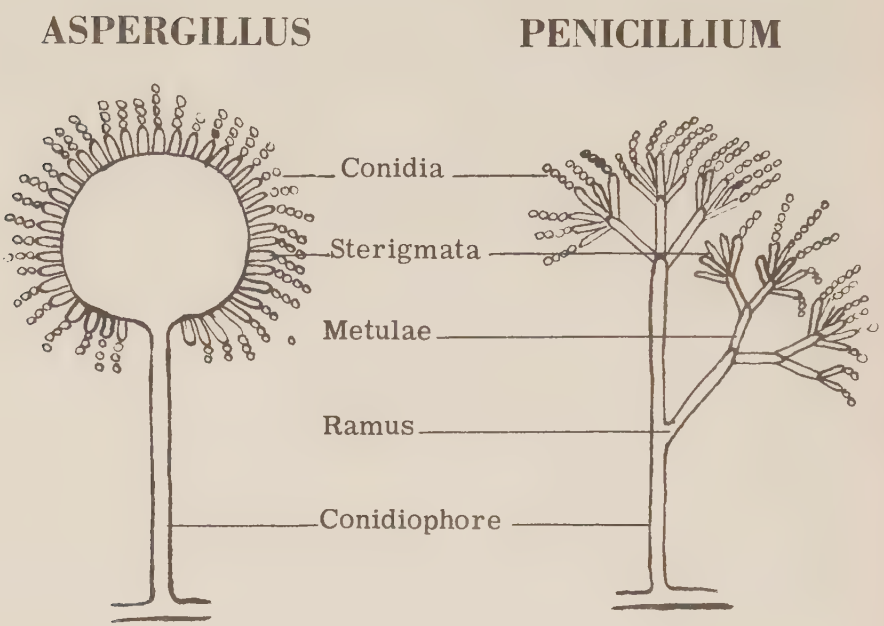
5. Thiourea (thiocarbamate) was an excellent fungicide in the concentrations ranging from 1.0 to 0.25 percent. It was effective either as an aqueous dip before the oil or in the oil as an emulsion.

6. Sodium pentachlorophenol was only partially effective in 1.0, 0.5, and 0.25 percent concentrations. Because of its irritating effect on the respiratory tract and a slight odor in the broken out eggs, it was considered unsatisfactory as a fungicide for eggs.

7. Alkyl dimethyl benzyl ammonium chloride was an excellent fungicide for use in processing oil, using 0.1 to 0.01 percent concentrations.

8. Sodium propionate, using a 4 percent aqueous solution, was found to be effective in inhibiting mold growth if it was used as a dip followed by oil processing.

APPENDIX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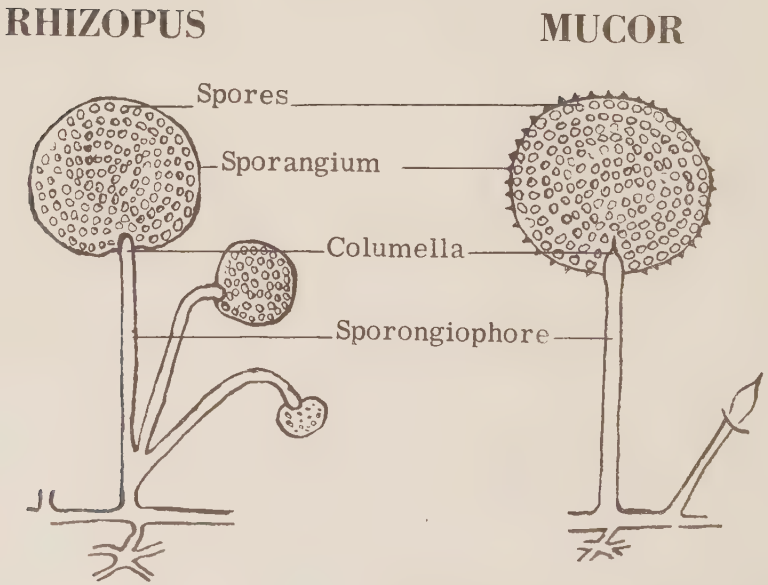
PENICILLIUM
Conidia
Sterigmata
Metulae
Ramus
Conidiophore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Spores
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Columella
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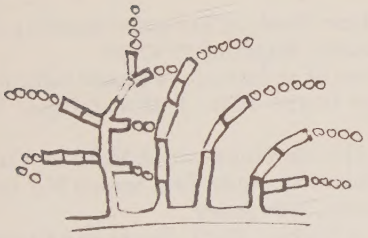
TRICHOTHECIUM

ALTERNARIUM

OIDIUM



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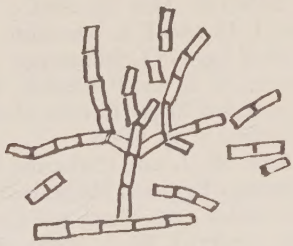

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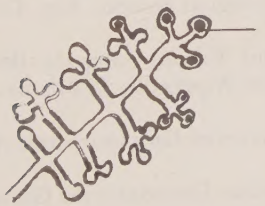

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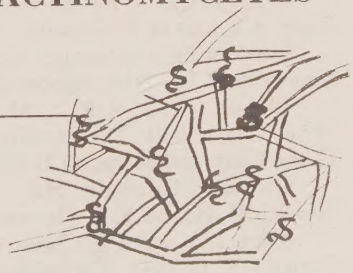

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